Nature of Interaction between

Metal Nanoparticles(Ag)

&

Bacterial Cell (E.Coli)

A Project Report Submitted in Partial Fulfilment of The Requirements

For The Degree in

Bachelor of Technology in Biotechnology Engineering

By

Deepika Rani Mittal
(Roll No.- 107BT013)

DEPARTMENT OF BIOTECHNLOGY AND MEDICAL ENGINEERING

NATIONAL INSTITUTE OF TECHNOLOGY ROURKELA

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CERTIFICATE

This is to certify that project entitled " NATURE OF INTERACTION BETWEEN SILVER NANOPARTICLES(Ag) AND BACTERIAL CELL (*E. Coli*)" submitted by DEEPIKA RANI MITTAL (Roll No. – 107BT013), in partial fulfilment of the requirements for the award of Bachelor of Technology in Biotechnology Engineering at National Institute of Technology, Rourkela (Deemed University) is an authentic work carried out by her under my supervision and guidance.

To the best of my knowledge, the matter embodied in the Project report has not been submitted to any other University/Institute for the award of any Degree or Diploma.

Date:
Place: Rourkela

(Prof. Subhankar Paul)
Department of Biotechnology & Medical Engineering
National Institute of Technology, Rourkela
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Last, but not the least I cannot forget to thank my friends at NIT Rouekela who were a great moral and practical support during my work.

Deepika Rani Mittal
(107BT013)

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Abbreviations

gm	**Gram**
ml	**Millilitre**
%	**Percentage**
hr	**Hour**
MBC	**Minimum Bactericidal Concentration**
min	**Minute**
C	**Centigrade**
Ag	**Silver**
LPS	**Lipopolysaccharide**
OD	**Optical Density**

ABSTRACT

In the present investigation, we demonstrated the nature of interaction between silver nanoparticles and *E.Coli* bacterial. Stable silver nanoparticles (NP) were prepared by green synthesis (Catharanthus plant extract) method and characterized by UV-Vis spectrophotometry, SEM and DLS (Dynamic Light Scattering) particle size analysis. The antimicrobial activity of silver nanoparticles was monitored against *Escherichia coli* (Gram negative bacteria). Nutrient broth solutions were used to culture the Escherichia coli and silver nanoparticles of different concentrations were added to the bacterial culture solution to monitor toxicity of NP and simultaneously to investigate cell-NP interaction. It was observed that *Escherichia coli* growth was inhibited at a NP concentration and 80μg/ml concentration NP was found to kill E.coli completely.

Key words: Silver nanoparticles, *Escherichia coli*, Antibacterial effect, Green synthesis

Chapter 1

Introduction and Objectives

Introduction

Nanoparticles show unique physical and chemical properties and have attracted much attention for their distinct characteristics. Their uniqueness arises specifically from higher surface to volume ratio. That's why they represent an increasingly important material in the development of nanotechnology and nanoparticles which can be used in numerous biological, physical, biomedical and pharmaceutical applications.

Resistance of bacteria to bactericides and antimicrobial agents has increased in recent years. Some antimicrobial agents are extremely toxic and irritant. Nano particles interaction with biomolecules and microorganisms is expanding the field of research. It is known from the centuries that Ag and Ag based compounds show antibacterial activities. Previous studies have shown that antimicrobial formulation in the form of nanoparticles can be used as effective bactericidal agents and this is safe and cost effective too. Thus the preparation, characterization, surface modification and functionalization of nanoparticles open the possibility of formulation of new generation of bactericidal agents.

Bacterial Cell

Bacteria have a very simple internal structure and no membrane bound organelles. Bacteria show a wide diversity of sizes and shapes, called *morphologies*. Bacterial cells are about one tenth the sizes of eukaryotic cells and are typically 0.5–5.0 micrometres in length. Beginning from the outer most structures and moving inward, bacteria have some or all of following structures:-

- Capsule (Polysaccharide membrane)
- Outer membrane(Lipid bilayer)

- Cell wall (Peptidoglycan)
- Periplasmic space
- Periplasmic membrane (Cytoplasmic plasma membrane)

Bacteria have different membrane structure on the basis of which these are classified as gram positive or gram negative. The structural difference between Gram positive and Gram negative bacteria lies in the organization of peptidoglycan which is the key component of membrane. The bacteria, we have used in our experiment is *E.coli,* which is Gram negative.

1. Structure and composition of gram negative cell wall

In electron micrographs, the gram negative cell wall appears multi-layered as shown in Figure 1.1. It consists of four layers:

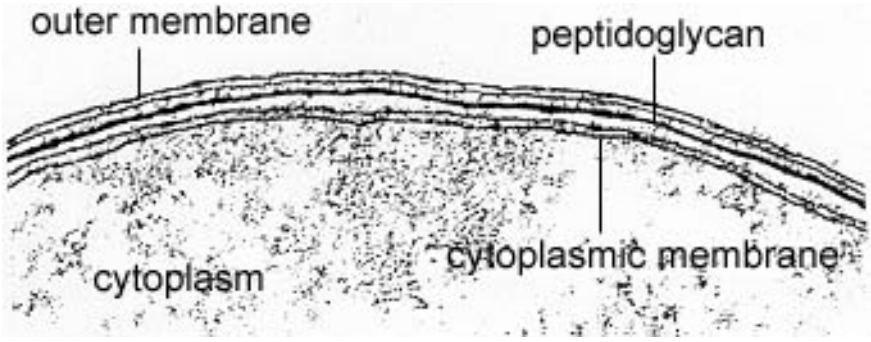


Figure 2.1: Electron Micrograph of a Gram-Negative Cell Wall

1. **A thin, inner wall composed of peptidoglycan:** Unlike the Gram-positive cell wall, Gram-negative bacteria have a thin wall consisting of a few layers of peptidoglycan. the peptidoglycan portion of the gram-negative cell wall is generally 2-3 nanometres (nm) thick and contains **just 2-3 layers of** peptidoglycan (see Fig. 1.2). Chemically, only 10 to 20% of the gram-negative cell wall is peptidoglycan.

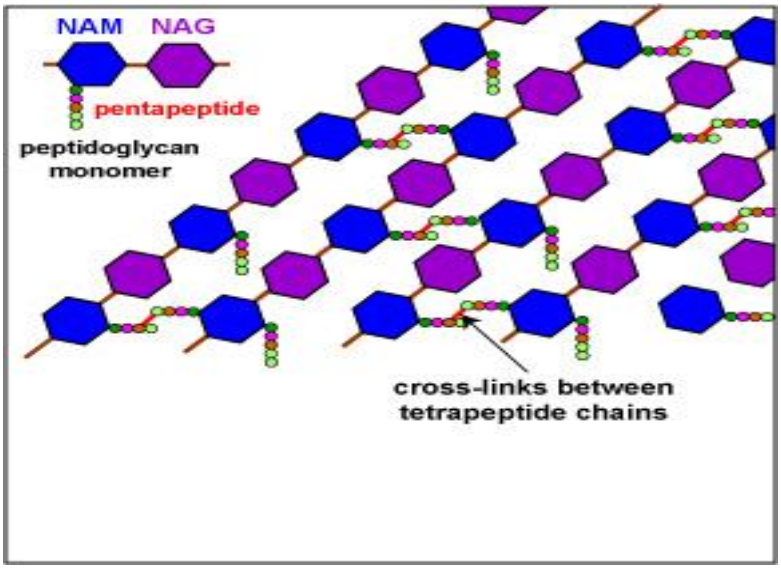


Figure 2.2: Structure of peptidoglycan: *E coli*

2. **An outer membrane:** The outer membrane of the gram-negative cell wall appears as a lipid bilayer about 7 nm thick. It is composed of phospholipids, lipoproteins, lipopolysaccharides (LPS), and proteins. Phospholipids are located mainly in the inner layer of the outer membrane, as are the lipoproteins that connect the outer membrane to the peptidoglycan (see Fig. 1.1). The lipopolysaccharides, located in the outer layer of the outer membrane, consist of a lipid portion called lipid A embedded in the membrane and a polysaccharide portion extending outward from the bacterial surface. The LPS portion of the outer membrane is also known as **endotoxin.**
3. The outer membrane of the gram-negative cell wall is studded with surface proteins that differ with the strain and species of the bacterium.
4. The periplasm is the gelatinous material between the outer membrane, the peptidoglycan, and the cytoplasmic membrane.

Bacteria do not have a nucleus bounded by membrane, and their genetic material is a single circular chromosome that is located in the cytoplasm, an irregularly shaped body known as the nucleoid. It contains the chromosomes with associated proteins and RNA.

2. *E. Coli* as a Model Organism

Escherichia coli commonly abbreviated *E.coli* named after Theodor Escherich is a gram negative rod shaped bacterium that is commonly found in lower intestine of warm blooded organisms (endotherms).

The *E.coli* bacterium (gram negative bacteria) was chosen because this is the most well understood bacterium in the world and it is an extremely important model organism in a number of fields of research, particularly genetics, molecular biology, and biochemistry. It is easy to grow under laboratory conditions and strains are very safe to work with. This organism grows quickly allowing many generations to be studied in a short time. Ecoli cells can double in number only after 20 minutes. A very large number of E.coli bacteria can be grown in small place, for example: millions of bacteria in a drop of broth. These are some important characteristics in genetic experiments which often involve selecting a single bacterium among millions of candidates, then allowing that to reproduce into high numbers again to perform additional number of experiments.

Many fundamental processes that are shared by living things are most easily studied by this simple *E.coli* bacterial model. *E. coli* has presented as a model for understanding the biology and metabolic mechanism of other bacteria. The ways in which *E. coli* interacts with the human body are very similar to the ways that other disease-causing organisms interact in many cases. Therefore, this model organism has allowed researchers to study the human health through an important organism.

Nanoparticles

Nanoparticles are particles that have at least one dimension in nano range that means 100 nanometres or less than that. A nanoparticle behaves as a whole unit in terms of transport and properties. Nanoparticles can be classified into Nanoclusters, Nanopowders, Nanocrystals, Nanotubes.

1. Why Nanoparticles???

The first and obvious question is why we are dealing with nanoparticles. Why they are so interesting? When their synthesis, characterization and handling are so complicated then why even bother to work with those extremely tiny structures? Now the answer is "Nanoparticles possess some extraordinary and unique properties which increase their importance and uses in Biology and Biochemistry".

Nanoparticles have a very high surface to volume ratio. This property makes them more reactive to some special molecules. This property can be used in the areas where high volume to surface ratio is requirement for successful experiment. Some nanoparticles show antibacterial activity against microbes and there high surface to volume ratio is important.

Nanoparticles are in range of 10-100nm and this is the size range of maximum human proteins and biomolecules. We can modify the properties of nanoparticles by controlling their shape, size and chemical properties that's why nanoparticles have attracted much attention. Since the synthesis of first nanoparticles their applications found their way into many different areas of science.

2. Why Ag Nanoparticles?

There are so many areas where Ag nanoparticles are proven to be effective. There are a number of applications where nanoparticles have been used successfully. A possible application of Ag nanoparticles is as a catalyst. Another application of Ag nanoparticles is as a real time optical sensor. The property which we have used here for our whole study was that Ag nanoparticles (NP) are effective in controlling and supressing the bacterial growth. The bactericidal effect of Ag can be divided into two groups: 1) Ag ions and 2) Ag nanoparticles [6]. Ag ions are positively charged atoms and Ag NPs are single crystals.

Experiments have already shown that Ag ions can make structural changes in cell membrane. The membrane of bacteria is consists of lot of sulphate containing enzymes and Ag ions interact with these sulphate groups and hence change the morphology by pacifying the enzymes. Because of this inactivation it is easier for Ag ions to penetrate the cell membrane and go inside the cell and continue to destroy the other parts of cell by interacting with sulphate groups located on the surface of enzymes. Ag ions can also interact with phosphorus groups of biomolecules. One example is the interaction of Ag ions and DNA which makes the bacteria unable to replicate itself.

The purpose of this study is not to show the antibacterial effect of Ag nanoparticles but try to find out the mechanism of action of Ag nanoparticles. The fact is that antibacterial effect of Ag ions is well known, however, the antibacterial activity mechanism of Ag nanoparticles is almost unknown. A number of experiments were performed in order to show the antibacterial effect of Ag nanoparticles.

3. Measurement and Characterization of Silver Nanoparticles

Various techniques are available for detection, measurement and characterization of Silver (Ag) nanoparticles. There is nothing like best method that can be selected, the method is chosen depending upon the method of synthesis, the amount of sample, the information required and the cost of analysis. Different techniques suit different type of samples. For example, some techniques require sample to be as an aerosol and some other techniques require sample to be as suspension or liquid.

Considerations before choosing a method:

1. The aim of measurement which include number, mass, particle size, surface area etc.
2. Type of sample required for analysis whether aerosol, or suspension or solid or liquid.
3. Amount of sample required and if one can discard the sample after analysis.
4. If the technique require sample to be prepared in a certain way.
5. Costs involved in the measurement technique.
6. How much time will the analysis take?

Measurement techniques are listed below:

1. Microscopy methods
    a. TEM (Transmission Electron Microscopy)
    b. HRTEM (High Resolution Transmission Electron Microscopy)
    c. SEM (Scanning Electron Microscopy)
    d. AFM (Atomic Force Microscopy)
2. PCS (Photon Correlation Spectroscopy)

    Photon Cross Correlation Spectroscopy is also available for measurements.
3. NSAM (Nanoparticle Surface Area Monitor)

4. CPC (Condensation Particle Counter)
5. DMA (Differential Mobility Analyser)
6. SMPS (Scanning Mobility Particle Sizer)
7. NTA (Nanoparticle Tracking Analysis)
8. XRD (X-Ray Diffraction)
9. ATFMS (Aerosol Time of Flight Mass Spectroscopy)
10. APM (Aerosol Particle Mass Analyser)
11. QCM (Quartz Crystal Microgravimetry)

Objectives of the thesis

1. Study the interaction between nanoparticles and *E. Coli* cell in vivo.

2. To study the mechanism of action of Ag nanoparticles on bacterial membrane.

Chapter 2

Literature Review

Silver (Ag) has been known to exhibit a countable toxicity to a wide range of micro-organisms and because of this reason Ag-based compounds have been used in many bactericidal applications. Ag compounds have also been used in the clinical field to treat burns and a number of infections. Several salts of Ag and their derivatives are commercially used as antimicrobial agents.

Electron microscopy showed the distribution and location of Ag nanoparticles, as well as the change in morphology of bacteria after treatment with Ag nanoparticles. Ag was found to adhere to the bacterial cells. Outer membrane of E.coli cells are constructed from highly packed lipopolysaccharide (LPS) molecules, which provide effective permeability barrier. The overall charge of bacterial cells at biological pH value is negative because of excess number of carboxylic groups which upon dissociation make the cell surface negative. The opposite charge of bacteria and nanoparticles are attributed for their adhesion and bioactivity due to electrostatic forces. It was stated that the binding of nanoparticles to the bacteria depends on the surface area available for interaction. Nanoparticles have larger area available for interaction which enhances the bactericidal effect then the large sized particles; that's why they impart cytotoxicity to the microorganisms [1].

Small size nanoparticles show better antibacterial activity because the decrease in volume increases the surface area hence increases the antibacterial activity. A Previous studies has shown that Ag nanoparticles of 100 nm were the least effective against bacteria because a lager dose is needed to reach the bactericidal effect (MBC). A direct comparison of the three Nano Ag sizes particles under the same dose range showed that size does matter. Discoveries in the past also have demonstrated that antibacterial properties of Ag nanoparticles are size dependant [2]. One publication have reported that Ag nanoballs having 12nm size show complete antibacterial characteristics against E.coli at 40µg/ml [3].

It has already shown that Ag nanoparticles mode of action is not the same as the mode of action exerted by the pre-existing antibiotics (β-lactamics, quinolones, aminoglycosides, trimethoprim-sulfamethoxazole, and vancomycin) [1]. Some studies have already shown that Both Ag nanoparticles and AgNO3 inhibited the growth of *E. coli* cells at the same concentration but the rate of inhibition appears to be slow with increasing concentration of AgNO3 compared to Ag nanoparticles. It was suggested that the MIC of AgNO3 is higher than Ag nanoparticles [4]. It was shown that 50 µg ml^{-1} of Ag nanoparticles solution cause 100 % growth inhibition but at the same concentration of 50 ug ml^{-1} AgNO3 inhibit only 80 % of growth during starting phase of treatment. It was also demonstrated that in case of 12 hr treatment using Ag nanoparticles, 100 per cent growth inhibition recorded from 30ug ml^{-1} to 50 µg ml-1, But AgNO3 showed lower performance, even in the 50 µg ml-1 concentration; more colonies grew on the plate [4].

Some researchers found that 10 µg ml-1 concentration of $AgNO_3$ and Ag nanoparticle was able to inhibit bacterial growth and create a zone of 0.8 cm and 1.7 cm respectively [4]. A study has shown that Nanoparticles of Ag do not have significantly different antimicrobial activity towards Gram positive and Gram negative bacteria [5]. Experimental evidence suggests that DNA loses its ability to replicate once the bacteria have been treated with Ag ions and Ag nanoparticles. The bactericidal activity of these nanoparticles depends on their stability in the culture medium too, since this imparts greater retention time for interaction of bacterium and nanoparticles [6].

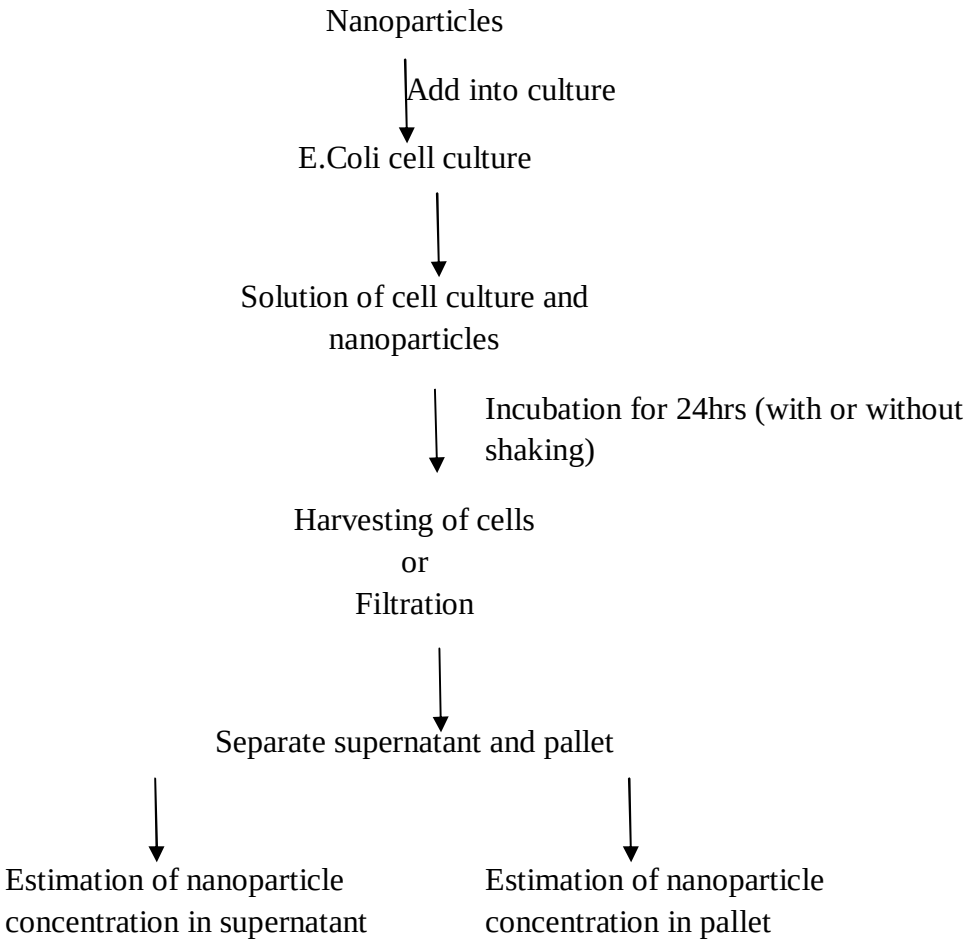
Previous studies suggests that nanoparticles toxicity also depends upon the pH and concentration of the nanoparticle suspension and toxicity is caused by the inherent properties of the Ag nanoparticles, and not by dissolution of the nanoparticles in solution and subsequent effects of the dissolved Ag [7]. Smaller size nanoparticles shows best

antibacterial activity, because of their smaller size nanoparticles can easily penetrate the cell and reach to the nuclear content of bacteria and they exhibit the largest surface area so area in contact with bacterial cell is the greatest. These directly mean that antibacterial activity of Ag nanoparticles can be enhanced by modifying the Ag nanoparticles [8]. Combination of Ag, copper and other nanoparticles may give rise to more effective bactericidal agents against mixed bacterial population [9] but detailed research, a lot of efforts and comparative study of bacterial strain specific variability is needed to find out the bactericidal efficacy of combine suspension of nanoparticles.

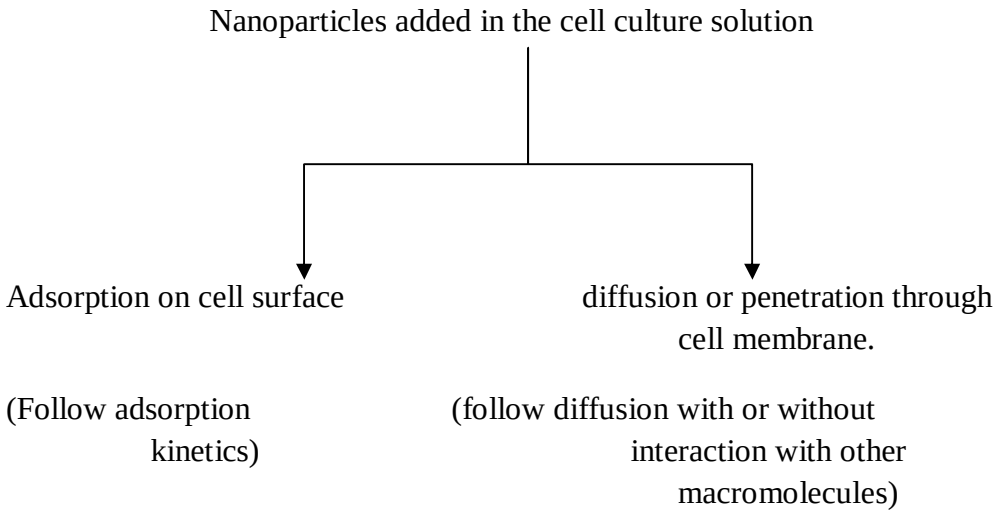
Chapter 3

Materials and Methods

1. Plan of work



2. Problem Analysis



3. Preparations:

1. Organism Preparation/Culture Preparation:

Fresh Colonies of Escherichia coli were obtained from NCCS (National Centre for Cell Science). Then E.coli was cultured into nutrient broth containing flasks for the further experiments.

2. Media Used:

Nutrient broth medium was prepared by dissolving 28 gm of nutrient agar in 1000 ml of mili-Q-water. The above solution was autoclaved subsequently at 121^{o}C, 15 lbs. for 30 min.

3. Synthesis of Ag nanoparticle formulation

Ag nanoparticles were synthesized according to the Green Synthesis Method described in the literature [21-26]. The Green Synthesis Method from catharanthus plant extract is described here.

Protocol:

Preparation of Catharanthus Plant Leaf Extract:

1. 10 gm of catharanthus plant leaves had cleaned and cut into pieces and taken into 100ml of distilled water.
2. Then the solution was boiled at $60^{o}C$ temperature for 20 minutes.
3. After that the solution filtered through 0.45µm and 0.22µm filters via filtration procedure
4. Filtered solution kept in $-4^{o}C$ refrigeration for further use.

Preparation of Ag nanoparticles solution:

1. 0.0169gm of 1mM $AgNO_3$ was taken and dissolved in 100ml of distilled water.
2. For 1 mM of $AgNO_3$, 2ml of catharanthus plant leaf extract was added and mixed.
3. Mixed solution was kept in incubation at room temperature for 20 minutes.
4. Finally yellowish brown colour was appeared indicating the presence of Ag nanoparticles.

Further tests were performed to confirm the presence of nanoparticles in the solution.

4. Different Conditions to apply nanoparticles in E.Coli culture:

1. Addition of Different nanoparticle concentration in same growth phase of cells for same incubation time without shaking.
2. Addition of same concentration of nanoparticles in different growth phases (lag phase, log phase, stationary phase and death phase) for same incubation time without shaking.

3. Addition of same concentration of nanoparticles in same growth phase for different incubation time without shaking.
4. Addition of nanoparticle in bacterial culture for same incubation time with shaking that means allowing further growth.

Different concentrations of Ag nanoparticles were added to the culture flask after 6 hrs of culturing (mid log phase). No nanoparticles were added to the control. Culture flasks were incubated in orbital shaker incubator for 24hrs, at 200rpm, at 30^{o}C. After incubation, culture flasks were stored at freezing temperature. Then culture was centrifuged at 6000rpm for 15min avoiding disruption. After centrifugation supernatant and pallet was separated, and then we washed the pallet with distilled water, and filtered both the solutions (supernatant and washed pallet solution) through filtration. Test was performed to check the presence of Ag nanoparticles in the supernatant and washed pallet solution.

4. Observation

1. Growth profile of bacteria:

To study the growth of bacteria inoculations were taken from fresh colonies. We tested the cultures for growth, after every 2 hrs of inoculation, we harvested the sample and measured the OD at 600nm. After 24hrs of incubation, cultural broth and nanoparticle solution was observed for turbidity.

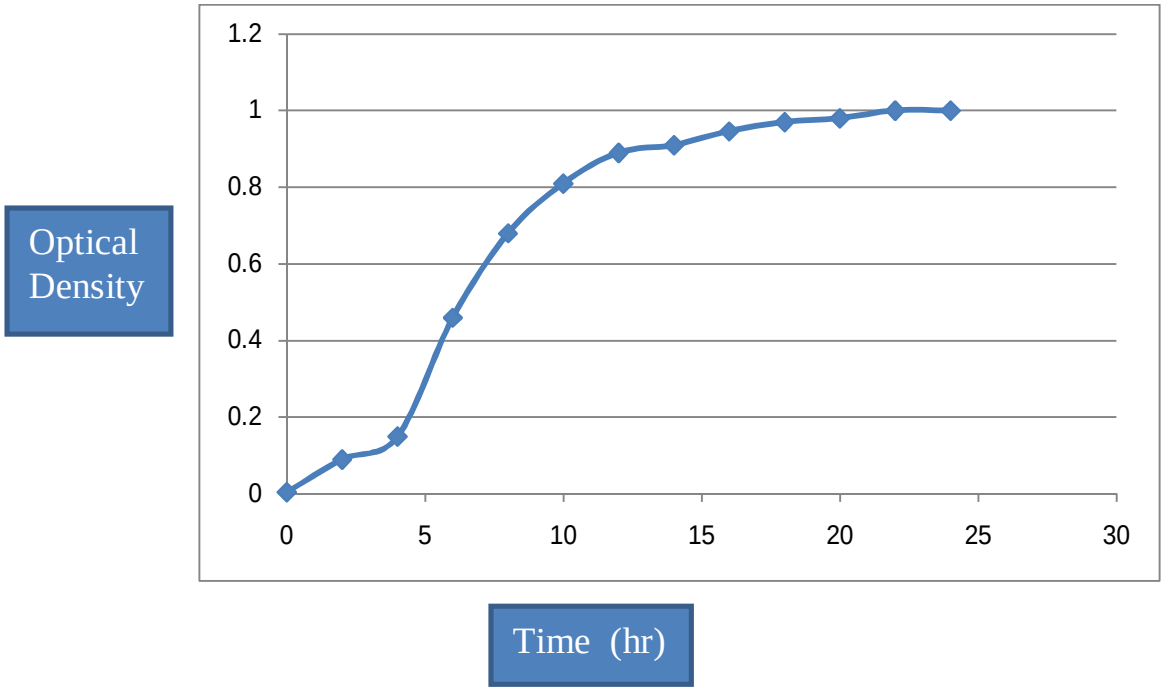


Figure 3.1: Growth Curve for *Escherichia coli*

As we can see from the above curve, mid log phase is after 6 hrs. That's why we added nanoparticles after 6hrs, that is in the mid log phase.

2. Detection of nanoparticles present in the culture solution

Supernatant and pallet were analysed for the presence of nanoparticles.

Sample (Supernatant)	Wavelength(nm)	Absorbance
Sample No. 1 (1ml of Ag nanoparticles solution)	--	--
Sample No. 2 (2ml of Ag nanoparticles solution)	--	--
Sample No. 3 (5ml of Ag nanoparticles solution)	437nm	0.357

Table 1. Spectrometry analysis of Supernatant

Sample (Pallet)	Wavelength(nm)	Absorbance
Sample No. 1 (1ml of Ag nanoparticles solution)	399nm	0.139
Sample No. 2 (2ml of Ag nanoparticles solution)	439nm	0.121
Sample No. 3 (5ml of Ag nanoparticles solution)	467nm	0.098

Table 2. Spectrometry analysis for pallet

Chapter 6

Results and Discussion

Results:

1. Visualization of colour:

Figure 6.1: Digital photograph of 1 mM $AgNO_3$ with catharanthus plant leaf extract showing the presence of Ag nanoparticles in the solution.

Figure 6.1 shows the presence of nanoparticles in the solution.

2. UV visible spectrophotometry of nanoparticles

The prepared aqueous solution (Figure 1) of Ag nanoparticles showed an absorption band at 392-450 nm as shown in figure 2, which is a typical absorption band of spherical Ag nanoparticles due to their Surface Plasmon.

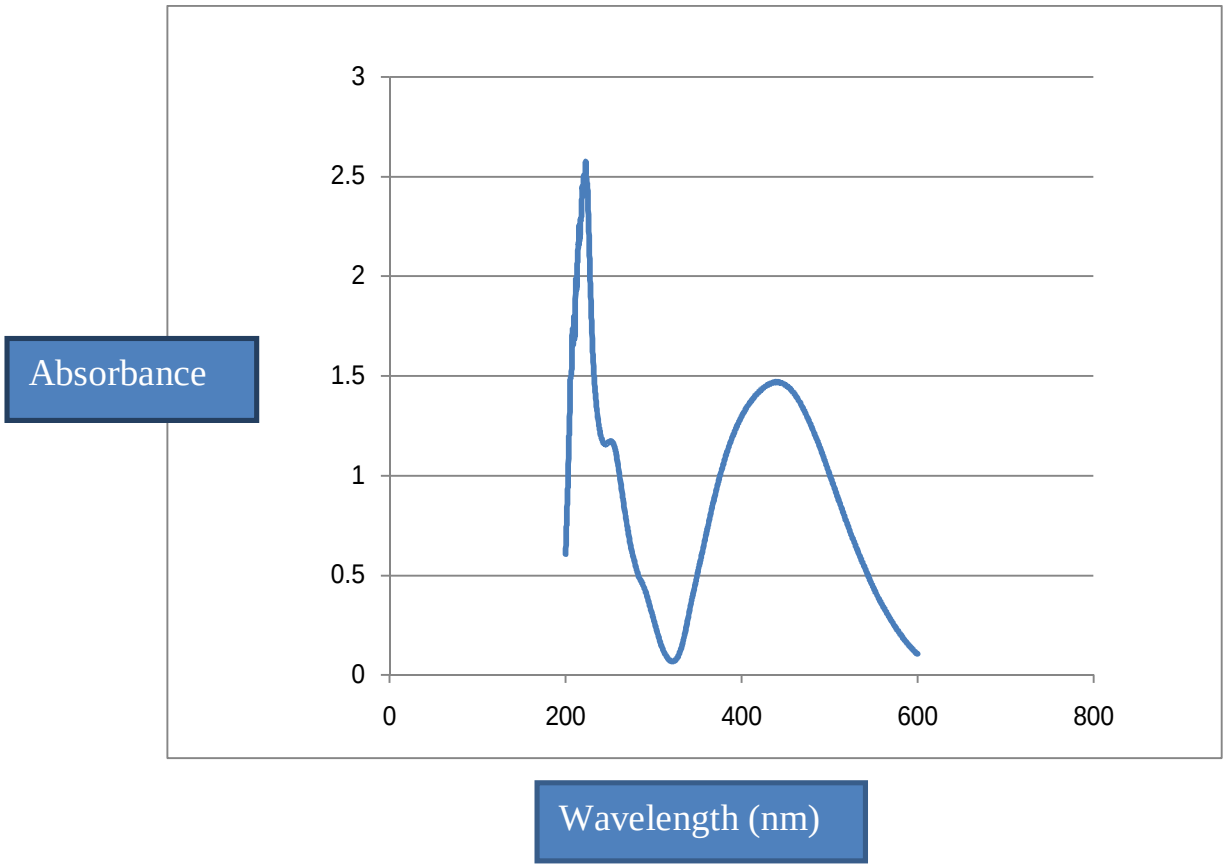


Figure 6.2 UV-Vis Absorption spectrum of Ag nanoparticles synthesized by treating 1mM aqueous $AgNO_3$ solution with catharanthus plant leaf extract

Figure 6.2 shows the UV-Vis spectra recorded from the reaction medium. Absorption spectra of Ag nanoparticle formed in the reaction medium has absorbance peak at 438nm, sharp peak indicates that particles are mono dispersed.

3. DLS Analysis of Ag nanoparticles

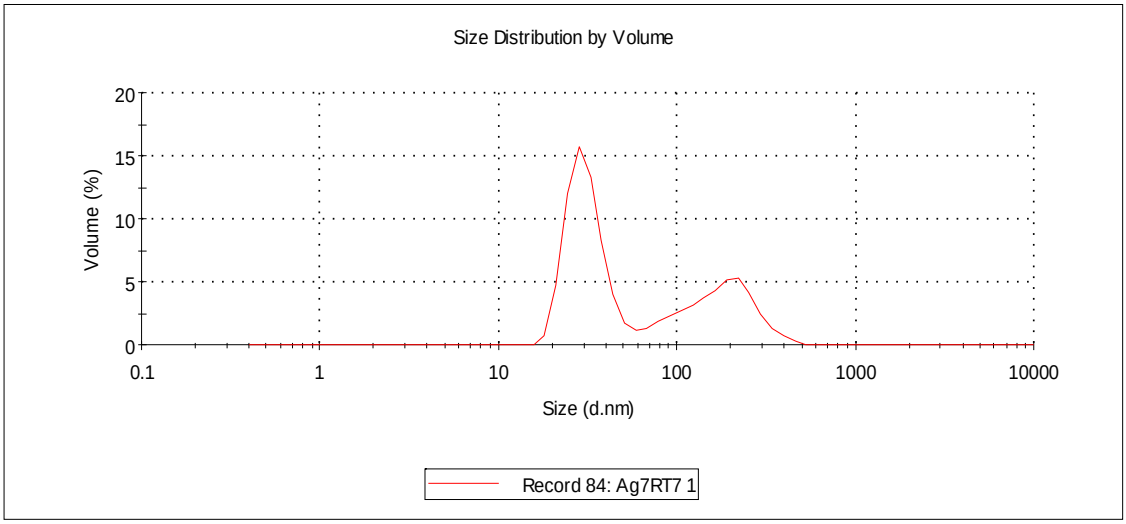


Figure 6.3: DLS Spectrum of nanoparticle solution

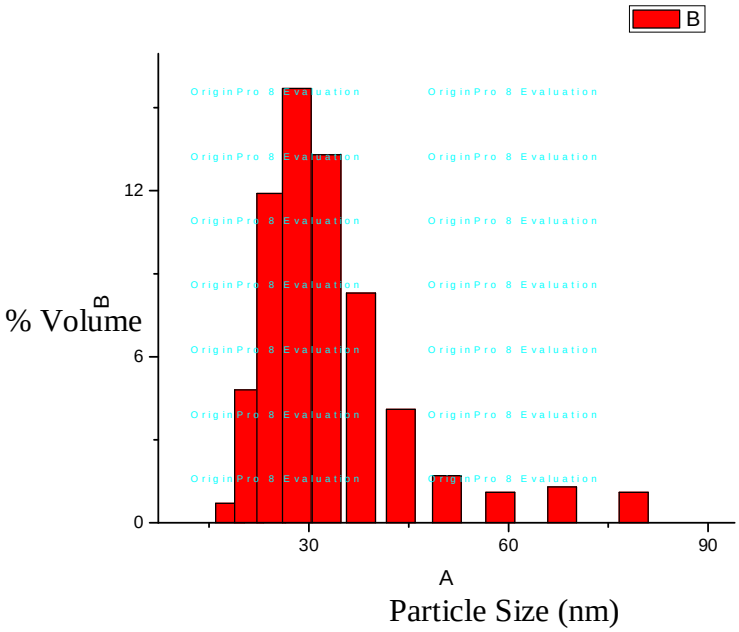


Figure 6.4: Particle size distribution of Ag nanoparticles synthesized by leaf extract

This figure 6.4 shows the graphical representation of average particle size distribution of Ag nanoparticles. From this graph this has been concluded that the average particles size of Ag nanoparticles synthesized by catahranthus leaf extract was 29 nm.

4. Scanning Electron Microscope:

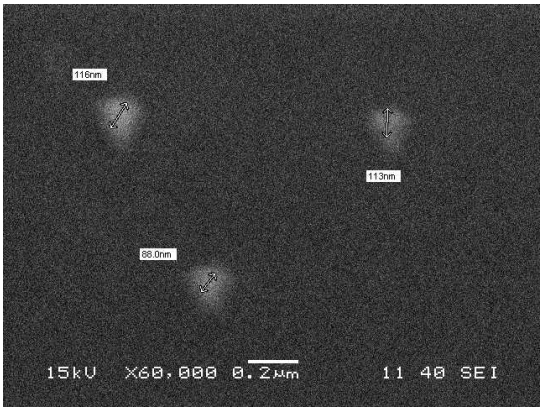


Figure 6.5: SEM micrograph of Ag nanoparticles synthesized by catharanthus leaf extract

Scanning Electron Microscopic (SEM) analysis was done using Jeol JSM-6480 LV SEM machine. Thin films of the sample were prepared on a glass slide by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the slide was allowed to dry by putting it under a mercury lamp for 10 min.

Figure 6.5 shows the SEM image of Ag nanoparticles synthesized by catharanthus leaf extract and 1mM $AgNO_3$ concentration.

5. Analysis of growth curve:

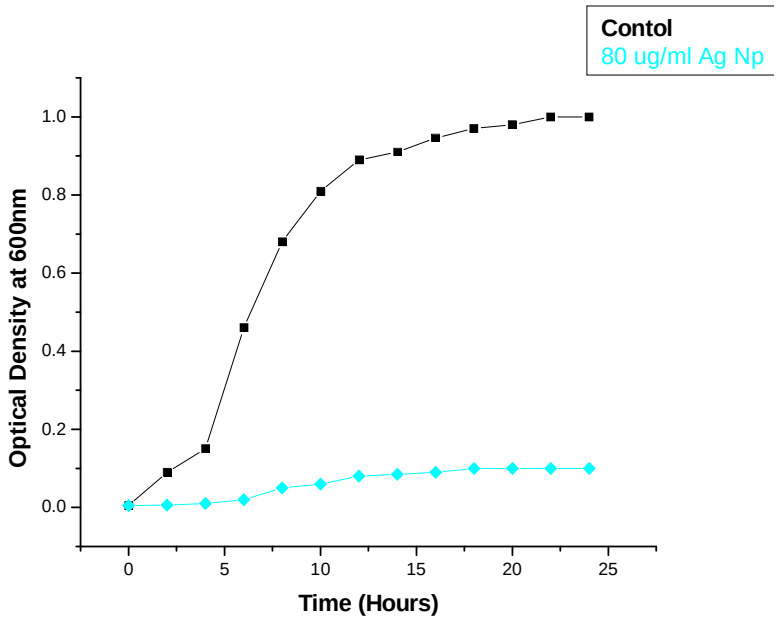


Figure 6.6: Growth Curve for *Escherichia coli* with or without nanoparticles

We can see here nanoparticles inhibited the growth of *E.coli* completely. No growth or less growth observed after addition of nanoparticles.

6. Detection of nanoparticles present in bacterial culture

Nanoparticles were detected in both the supernatant and pallet. Concentration of nanoparticles in each sample of pallet and supernatant was different depending upon concentration.

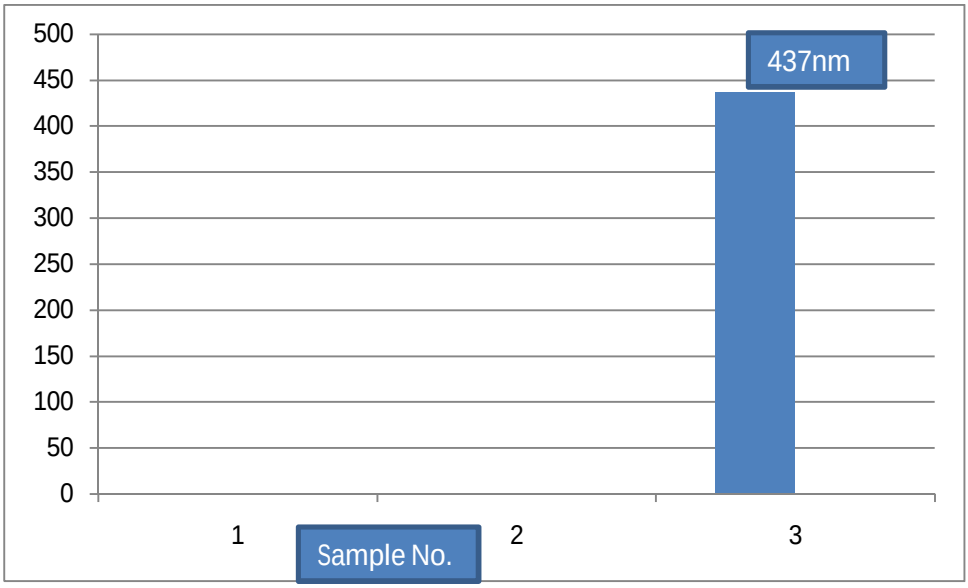


Figure 6.7 Absorbance of different samples of supernatant

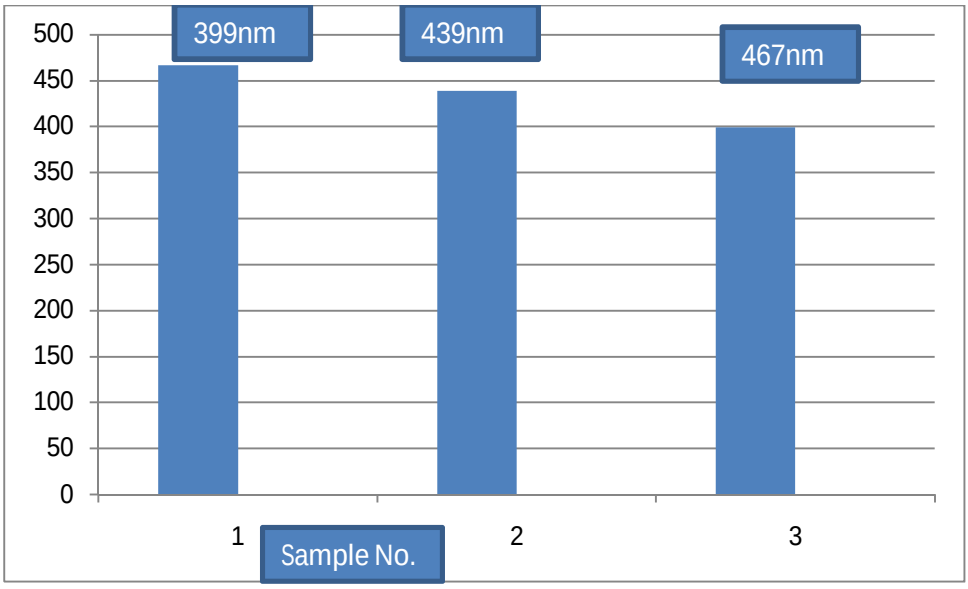


Figure 6.8 Absorbance of different samples of pallet

Discussion

We all know that Ag ions and Ag compounds exhibit antibacterial activity. Many investigators are trying to use other inorganic nanoparticles as antibacterial agents. These inorganic nanoparticles are advantageous over conventional chemical antimicrobial agents which we use. The main problem is that bacteria have developed resistance towards these types of antibacterial agents. That's where the requirement arises to develop or to search some new antibacterial agents. Generally the mechanism of antibacterial activity of such chemical agents depends on the specific binding with the surface receptor of bacteria and metabolism of agents into the microorganism. Alternative way for antibacterial activity is using Ag compounds. Many other researchers have already tried to check the activity of Ag ions or nanoparticles against microorganisms.

To use Ag in various fields against microorganisms, it is needed to prepare the Ag nanoparticles with cost effective methods and to find out the mechanism of antibacterial activity. In this report we demonstrate that Ag nanoparticles can be prepared cost effectively. Ag nanoparticles were synthesized by using green synthesis method from catharanthus plant leaf extract and characterized by UV-Vis spectrophotometry and DLS. Inhibition or antibacterial activity depends on the concentration of the Ag nanoparticle solution as well as on the CFU of bacteria that was used in the experiments. When Ag nanoparticles were tested against Escherichia coli, they effectively inhibited the growth. The mechanism of antibacterial effect of Ag ions on bacteria is partially known. Some studies have reported that the positive charge on the Ag ion is responsible for its antimicrobial activity through the electrostatic attraction between negative charged cell membrane of microorganism and positive charged nanoparticles.

Now the very obvious question in anyone's mind is how Ag nanoparticles acts as a antibacterial agent against *E.coli*.The mechanism by which nanoparticles penetrate into the bacteria is not understood completely, but studies suggest that when E.coli was treated with Ag changes took place in its morphology and produced a significant increase in its permeability affecting proper transport through plasma membrane, leaving the bacterial cells incapable of regulating transport properly through the plasma membrane, resulting into cell death. It was observed that nanoparticles have penetrated inside the bacteria and have caused damage by interacting with sulphur and phosphorus containing compounds such as DNA. Ag tends to have a high affinity towards such compounds. In our study it is considered that DNA may have lost its replication ability and cellular protein become inactive after treatment with Ag nanoparticles. Another reason would be the release of Ag ions from the nanoparticles which will have an additional contribution to the bactericidal efficiency of nanoparticles. Recently many publications have reported antibacterial effect of Ag nanoparticle but in this thesis the effect of Ag nanoparticles on E.coli was studied. Due the unique and different properties of nanoparticles, we can use nanoparticles as a reasonable alternative for development of new bactericidal agents.

Assumptions:

- Two mechanisms of interaction is perhaps possible:
    1. Adsorption on cell surface
    2. Penetration inside cell membrane.
- If the size of nanoparticles is greater than 10 nm then nanoparticles will be adsorbed on the surface of the cell and cause the bacteria death due to long term accumulation of nanoparticles.
- If the size of nanoparticles is less than 10 nm then nanoparticles will get through the cell membrane.

We choose to take nanoparticles in the range greater than 10nm to check how much minimum concentration is needed to start the absorption ar adherence of nanoparticles on to the *E.coli* bacteria. As we can see from Table No. 1 and Table No. 2 when low concentration was applied to culture, no nanoparticles were observed in the supernatant solution but there were nanoparticles in the washed pallet, this means that nanoparticles applied onto culture was adsorbed. When high concentration or more amount of nanoparticles were added to the culture, nanoparticles were observed in both the solutions (Supernatant and Washed pallet solution), this means not all the nanoparticles added were adsorbed, some remained suspended in the Culture solution (supernatant). We can see that rate of adsorption is slowing down while adding the more number of particles.

From the Figure 6.6 we can see that nanoparticles are inhibiting the growth of bacteria. After addition of nanoparticles no growth or almost zero growth was observed. This curve is explaining the antibacterial effects of Ag nanoparticles significantly.

Chapter 7

Conclusion And Future Work

Conclusion:

This study showed that interaction of Ag nanoparticles with the bacterial cell and the mechanism of interaction of nanoparticle in the bacterial cell along with its antimicrobial activity. The Ag nanoparticles synthesized by cost effective Green Synthesis Method have shown excellent antibacterial activity.in this interaction the Ag nanoparticle that are having lesser size with less concentraction will penetrate inside the cell and were interacted intracellularly through absorption process and showed high inhibition of growth by arresting the metabolic mechanisms and those having larger size of nanoparticles were interacted extracellularly with high concentraction which shows less inhibition growth. So from these studies it was concluded that the interaction of nanoparticles with bacterial cell is varied based on their size and concentration.

Future work:

Further studies and research can be conducted in the following directions:

- To develop a model depending upon the Size and concentration of nanoparticles, pH of solution, time for which nanoparticles are allowed to interact with bacteria.
- To find the number of nanoparticles entered into the bacteria body to kill them.
- The same experiment can be done on a number of bacteria and a general modelling equation can be generated from the data and experiments.

Chapter 8

Instruments used

1. UV-Vis Spectroscopy:

Ultraviolet-visible spectroscopy (also known as **ultraviolet-visible spectrophotometry)** refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and near-UV and near-infrared (NIR) ranges for absorption spectra. The absorption or reflectance in the visible range directly affects the colour of the chemicals or presence of particles involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions and thus showing different absorption spectrum for different molecules. This technique is complementary to fluorescence spectroscopy, in the fluorescence spectroscopy deals with transitions from the excited state to the ground state, while absorption spectroscopy measures transitions from the ground state to the excited state.

This method is used in a quantitative way to determine the concentrations of an absorbing species in solution. Measuring the concentration follows the Beer-Lambert Law. The Beer-Lambert Law is useful for characterizing many compounds but this does not hold as a universal relationship for the absorption and concentration of all substances.

The Beer-Lambert Law:

$$A = \log_{10} \frac{Io}{I}$$

$$= \epsilon \cdot c \cdot L$$

Here, A is the measured absorbance**,**

Io is the intensity of the incident light at a given wavelength**,**

I is the transmitted intensity,

L the path length through the sample, and

c the concentration of the absorbing species.

ε is a constant (known as) the molar absorptivity or extinction coefficient For each species and wavelength this constant is a fundamental molecular property in a given solvent, at a particular temperature and pressure.

The instrument used in ultraviolet-visible spectroscopy is called a **UV/Vis spectrophotometer**. It measures the intensity of light passing through a sample (*I*), and compares it to the intensity of light before it passes through the sample (I_o). The ratio I / I_o is called the **transmittance**, and transmittance is usually expressed as a percentage (%T). The absorbance *A*, is based on the transmittance:

$$A = -\log(\%T / 100\%)$$

This spectrophotometer can also be configured to measure reflectance. In that case, the spectrophotometer measures the intensity of light reflected from a sample (*I*), and compares it to the intensity of light reflected from a reference material (I_o). The ratio I / I_o is called the **reflectance**, and is usually expressed as a percentage (%R). A complete spectrum of the absorption at all wavelengths of interest can often be produced directly by a more sophisticated spectrophotometer.

2. DLS (Dynamic Light Scattering)

Dynamic light scattering is also known as photon correlation spectroscopy or quasi-elastic light scattering. This is a technique in physics, which can be used to determine the size distribution profile of small particles in the suspension or polymers in solution. It can also be used to probe the behavior of complex fluids such as concentrated polymer solutions.

When light hits small particles the light scatters in all directions (Rayleigh scattering). so long as the particles are small compared to the wavelength. If the light source is a laser (monochromatic and coherent), then one observes a time-dependent fluctuation in the scattering intensity. These fluctuations are due to the fact that the small molecules in solutions are undergoing Brownian motion and so the distance between the scatterers in the solution is constantly changing with time. This scattered light then undergoes either constructive or destructive interference by the surrounding particles and within this intensity fluctuation, information is contained about the time scale of movement of the scatterers. There are several ways to derive dynamic information about particles' movement in solution by Brownian motion. One such method is dynamic light scattering, also known as quasi-elastic laser light scattering.

Chapter 9

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